THE EGG LAYING HABITS OF CALIFORNIAN ANOPHELINES *

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During the months of May, June and July, 1920, the writers were stationed at Vina, Tehama County, California, in the central part of the Sacramento Valley, where a temporary summer laboratory was established for the purpose of investigating a number of problems concerning malaria and anophelines, particularly their egg laying habits, with which this paper deals. The information herein published will in a measure supplement, confirm and correct previous observations in this field.

The species dealt with were *Anopheles occidentalis* D. & K., *Anopheles punctipennis* Say and *Anopheles pseudopunctipennis* Theobald. The synonomy of the first mentioned mosquito, *A. occidentalis* D. & K., is somewhat obscure in that it was separated from *A. quadrimaculatus,* which it closely resembles, by Dyar and Knab in 1906, and its range was listed by them as "western United States, from Southern California to Alaska, eastward thru Canada to Maine." However, Howard, Dyar and Knab (1917) in their monograph point out as an additional note that Say's type specimen for *A. quadrimaculatus* has as its locality, "Northwest Territory," which discovery makes the four-spotted anopheline of the Pacific coast, *Anopheles quadrimaculatus,* submerging their name, *Anopheles occidentalis,* and making the Eastern species *A. guttulatus* Harris. This revised synonomy has not come into general use, however, and with the above explanation offered to avoid future confusion we shall use the name *quadrimaculatus* to refer to the Pacific coast species.

The mosquitoes used in the observations herein described were captured in shell vials at a number of stations in the vicinity, such as bridges, stables, outhouses and dwellings that were visited regularly each day. The captured mosquitoes were transferred to wide mouthed pint jars partly filled with water and covered with bobbinet. The jars were placed in rows on a glass shelf supported at its corners about six inches above the surface of a laboratory table. Thus elevated and resting upon the glass shelf an incandescent electric lamp could be placed directly beneath the jars for purposes of illumination and greatly facilitated observations from above, particularly in counting the eggs.

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The water used in the jars at all times was taken from a deep well and showed a uniform alkalinity of 1840 parts per million expressed in parts of $CaCO_3$. The fauna and flora of the water was not determined but was practically negligible owing to the great depth of the bored well. Contamination that ensued later was undoubtedly uniform as all jars were concentrated in a small area not over 12 by 28 inches.

The temperature of the room in which these observations were made was taken on a recording thermometer. The record of this instrument showed at the expiration of the work that the maximum and minimum temperatures were 88° F. and 66° F., respectively, and that the average of each was 76° F. and 68° F. Here again there could have been no variation in the temperatures of the several jars.

Time of Deposition.—A total of sixty-five layings was recorded from May 17 to July 11. On thirty occasions we were able to obtain the exact or approximate time of oviposition. Of these, thirteen layings were made between nine and eleven in the evening, nine between eleven and daybreak, seven between sunset and nine p. m. and four during the afternoon. It must be understood, however, that artificial conditions may have seriously influenced these findings when it is considered that the light was never as intense in the laboratory as it would have been out-of-doors, that wind exercised little influence, and that the temperature curve showed a lag of approximately two hours as compared with out-of-doors as well as a distinct moderation.

The factor or factors governing the time of egg deposition are not known, but it would seem that light, temperature, humidity, and wind are probably important considerations. It is interesting in this connection that *Anopheles punctipennis* repeatedly deposited eggs in the full glow of a 40 watt tungsten lamp at a distance of about seven inches from the egg laying insect. It is safe to assume, however, that in these instances the insects oviposited in spite of the light conditions rather than because of them. That diffuse light or darkness is the normal condition during which eggs are deposited, is well illustrated by the fact that on June 3 the lights were left on from dusk until ten p. m., during which time there had been no oviposition. At that time the lights were turned off for ten minutes and at 10:10 when the lights were switched on it was noted that three Anophelines had deposited eggs, none of them resuming until the light was again extinguished. The daylight records of oviposition appearing in our notes are without exception for overcast, humid days when the light intensity in the house approximated that normally occurring at dusk. The range of temperature between different oviposition periods was so great as to suggest but slight effect at best unless the stimulus should arise from

a change of temperature as from warmer to cooler and vice versa. Unfortunately, no humidity records were kept. Further work with this factor in consideration seems to offer a promising field.

Method of Oviposition.—Owing to the reluctance of *A. quadrimaculatus* to oviposit in the presence of light and the scarcity of *A. pseudopunctipennis* our observations regarding the actual process of deposition are limited to but one species, *A. punctipennis.* The only reference in the literature concerning this process that we have been able to locate is that of Kerschbaumer cited by Nuttall and Shipley (1902). Nuttall writes: "With the exception of Kerschbaumer (1901) nobody has claimed to have observed the process of oviposition. He witnessed the process but once in Anopheles, . . . he does not, however, describe the process (excepting in so far as he says the insect rested directly upon the water)."

On the evening of June 4 a specimen of *Anopheles punctipennis* was seen to behave in a rather excited manner, resting for a few moments on the surface of the water and then flying to the bobbinet or sides of the jar, remaining in each position only a few seconds. She finally came to rest for several minutes on the surface and assumed a position with the abdomen more or less parallel with the surface of the water, the wings held in the normal position with relation to each other but elevated at least the width of the body above the abdomen, the posterior end of which, comprising the last two segments, was tilted upward slightly. All six tarsi rested on the surface of the water, the middle pair being lifted above the body from time to time.

At 9:46 p. m. the first egg was deposited. This was accomplished by a rather nervous jerk of the abdomen following which an egg was seen to be protruding in a vertical position from the abdomen with its convex side directed to the rear. This position was held for four seconds when another convulsive downward twitch freed the egg from the abdomen, and as the latter was returned to its former position, another egg protruded and slipped instantaneously into the vertical position as the tip of the abdomen regained its original attitude. This procedure was continued for 19 minutes until a total of 174 eggs had been deposited. The deposition of the individual eggs took place at remarkably regular intervals of from six to seven seconds. During the entire operation the female remained motionless except for the monotonus jerking of the abdomen. At the conclusion of oviposition the mosquito remained without changing position for eight minutes, after which she slowly moved off to the side of the jar, scattering the eggs with her legs as she went. Numerous statements, based evidently on the remarkable patterns assumed by the eggs on the surface, appear in the literature regarding the method of placing the ova. Grassi,

quoted from Nuttall and Shipley (1901), stated that the eggs of *A. maculipennis* were deposited in pontoons, while those of *A. bifurcatus* were laid in star shaped patterns. In the above example, however, and in many subsequent observations of the same species, the eggs were seen merely to pile up in a heap beneath the insect, toppling over as the mass became top heavy and arranging themselves in various patterns dependent upon mutual adhesion and surface tension. At the time of deposition the eggs are pearly white, becoming progressively yellowish, then darker, until at the end of about thirty-five minutes the color becomes distinctly leaden, and in about forty-five minutes they appear dull black and under the microscope are a rich chitinous brown.

Number of Eggs Deposited.—Grassi, quoted from Nuttall and Shipley (1901), states that *A. maculipennis,* the European representative of our *quadrimaculatus-guttulatus* group, deposits 100 eggs, while Hindle (1914) dealing with the same species places the number at from 40 to 100. Howard (1900) referring merely to Anopheles (no species given) also gives the range from 40 to 100. Our observations point to a considerably larger number per laying. It is impossible at the present stage of our investigations to estimate the total number of eggs laid during the life of an Anopheline as we have not been able to start our series with bred females. Our experimental insects were invariably captured specimens concerning whose previous oviposition history we, of course, have no record.

Twenty-nine specimens of *A. quadrimaculatus* deposited thirty layings totalling 6,282 eggs, in lots ranging from 140 to 315 eggs each, bringing the average per laying to 209 eggs. Thirty-three females of *A. punctipennis* in thirty-three layings, ranging from 83 to 321 eggs each, deposited 6,700 eggs; making the average per laying 203 for this species.

Our records of oviposition in *Anopheles pseudopunctipennis* are extremely limited. We were able to obtain only four females during the course of the work. Of these, two oviposited, one a total of 157 eggs and the other but 55, bringing the average to 106 eggs per female.

Considering the females of all species under observation, 38.4% oviposited in captivity. *Anopheles occidentalis* females showed an oviposition percentage of 48.3%, *A. punctipennis* 31.2% and *A. pseudopunctipennis,* based on only four specimens, 50%. These figures are not, of course, indicative of what the particular species may do in natural surroundings as our specimens, as already stated, were captured females, unfed in captivity in the majority of cases and whose opportunities for feeding before capture were unknown.

It is pertinent at this point to make a statement regarding the number of batches of eggs deposited. In the course of the work we had several cases where the females oviposited on two consecutive nights. In such cases the two layings were recorded as one. One specimen, *A. quadrimaculatus,* no 61, deposited two true batches of eggs. In this case the female was captured under a bridge on June 9 and during the afternoon of June 12 she deposited 218 eggs. On June 13 she was given a meal of human blood and on June 19 deposited 140 eggs, dying on June 20. Both batches of eggs were hatched on the morning of June 15 and 21, respectively. On numerous occasions, dissections of females that had oviposited and been fed showed the ovaries completely filled with well developed eggs. Numerous observers have stated that Anopheles may deposit several batches of eggs with a single fertilization and a blood meal for each complement of eggs. The exact number of batches and the length of time over which they are deposited needs further observation. Accurate information on this particular point is highly desirable and might change present emphasis in control work.

The hazards of life in captivity probably affected oviposition, many dying thru accident by getting "spraddled" in the water before they were ready to oviposit. Fully fifty per cent. of those dying without ovipositing showed the presence of complements of well developed eggs upon dissection. The average length of life for unfed *A. quadrimaculatus* in captivity, disregarding their probable length of life before capture, was 4.5 days and for the fed specimens 8.5 days. For *A. punctipennis,* the length of life for unfed specimens under the same conditions was 4 days and with food, 6.3 days.

Morphology of the Eggs.—In comparison with the extensive work that has been done on the morphology and classification of the other stages of the anopheline life cycle, little has been done with the eggs. The ease of classification (at least for the three Californian species) by means of egg characters recommends this line of study to workers in other localities. The characters found to vary in such a manner as to make identification simple, are length of the egg, and position and length of the float. The consideration of one or more of these factors is sufficient to place the egg of local species correctly, but in a larger group it would be necessary to utilize other characters, such as the "frill" of Stephens and Christophers (1908), a feature omitted in most of the illustrations of anopheline eggs, which encircles the flat or upper side of the egg, the formation of the floats, or the reticular membrane enclosing the egg.

These authors classify anopheline eggs into three groups: 1. those with the lateral floats not touching the margin; 2. those whose lateral

floats overlap the upper side of the egg; 3. those without floats. According to this classification *A. quadrimaculatus* and *A. punctipennis* fall in the second class and *A. pseudopunctipennis* in the third.

The egg of *A. quadrimaculatus* is fusiform, slightly rounded at each end, and tapering to the extent that one end is slightly broader than the other. The upper surface is flattened with a slight longitudinal concavity while the lower surface is broadly convex, the convexity becoming more pronounced at the broad end of the egg. The upper surface is granular, bordered by a laterally striated frill 16μ in width, except at the floats, while the lower surface shows, under proper light, a silvery reticulation. Medianly placed are two roughly oval lateral floats, each divided in a majority of cases into twelve scalloped compartments. The larger part of the area covered by these floats is on the lateral faces of the egg, but they project dorsally over the margins which are described as "gunwales" rather aptly by one author who

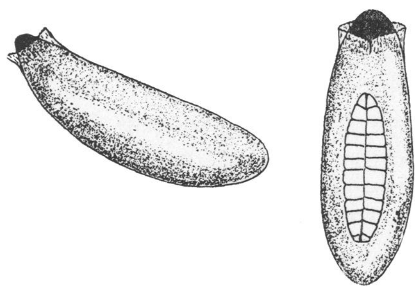

Fig. 1.—Illustrating the egg of *Anopheles pseudopunctipennis* Theobald. At the left, lateral view, and at the right, dorsal view.

likens the egg to a boat. The eggs range in length from 592 to 656μ. The floats vary in length from 144 to 224μ.

The eggs of *A. punctipennis* resemble those described above with these exceptions, the upper flattened surface is distinctly concave longitudinally, the floats are decidedly wider, projecting dorsally over the margins to the extent that they more nearly meet on the dorsal median line than those of *A. quadrimaculatus*. The floats also include approximately eighteen scalloped compartments each and extend along the sides for slightly more than one-half the entire length of the egg, while in *A. quadrimaculatus* the floats extend for only one-third the length. The range in length of the eggs of *A. punctipennis* extends from 544 to 576μ while the float length remains fairly constant at 320μ.

In *Anopheles pseudopunctipennis* is found a peculiar specialization represented in the general characters of the egg of *A. turkhudi* Liston, which is placed by Stephens and Christophers (1908) in class three of their table as lacking floats, altho vestiges are present. The eggs

are shorter than either of the two already mentioned, ranging from 512 to 528μ. The upper surface is nearly flat, showing little concavity longitudinally altho the lower surface shows a marked convexity. Both ends of the egg are rounded, one being considerably broader than the other. The floats are represented by a fusiform closely appressed area, approximately 270μ long, lying on the dorsal side of the egg and nearer the blunt end. This area is divided medianly by a line which is assumed by the authors to be the line of contact of the two floats that have been forced up from the sides. Lateral lines mark off each longitudinal half of the area into twelve sections representing the twelve original compartments of lateral floats. This area is so appressed that its position is not distinguishable from a lateral view.

Near the narrow end of the egg the membranous covering flares out from the body of the egg to form a translucent, striated collar which completely encircles the end, with the exception of a triangular incision down the dorsal median line in a manner which reminds one of an "oversized dress collar" (Fig. 1). The egg hangs at an angle in the water, supported by surface tension on this "collar." The larvae, however, unlike those of *Anopheles turkhudi,* whose eggs those of *pseudopunctipennis* resemble, retain the horizontal position at the surface of the water.

Selection of Breeding Places.—Much has been written regarding the type of breeding places frequented by various species of Anophelines and experienced observers in this field are able to forecast with a high degree of accuracy the species that they will find breeding in a given situation. This intuition is almost impossible to analyze and attempts to work out the ecology have yielded as yet only partial explanations. Disregarding the causes that make particular pools acceptable or unfavorable for the life of the larvae, there remains a fundamental question on which the whole study depends. This is, the determination of whether the selection of the particular pools is due to selective oviposition on the part of the female or the inhibiting effects, chemical or biological, upon the larvae present in some pools which are unfavorable to the species under consideration.

In the course of our work we found pools from which there constantly emerged, in the one case *A. quadrimaculatus* and in the other *A. punctipennis* with no mixture of the species. These pools were therefore classified as *quadrimaculatus* pools or *punctipennis* pools. Eggs of each species were "planted" in the pools hitherto inhabited only by the larvae of the other species and their development observed. In order to accomplish this under the most natural conditions, "lug" boxes with bobbinet coverings substituted for bottoms were inverted in the pools, supported a little from the bottom by stakes

and reaching an equal distance above the surface to prevent overflow. By supporting them a little from the bottom it was hoped that the natural enemies might successfully enter and that the enclosed water would partake of all the conditions prevalent in the pool and still not allow the escape in any appreciable numbers of the surface feeding larvae. Through an opening in the bobbinet covering, the eggs were gently washed on the surface of the water in the box. Unfortunately our boxes were of necessity located in pools subject to the rise of a creek, an occurrence that happened several times in the night owing to thunder storms in the mountains and the unexpected flow of unused irrigation water. Due to this contingency all of our boxes with the

Fig. 2.—Showing manner in which lug boxes were placed in a typical *A. punctipennis* pool to determine suitability of this pool to other species of anophelines.

exception of one set were found on one or more mornings to be awash, rendering their results problematical. One set, however, was conducted under optimum conditions. A pool, six by twenty-five feet in area known in our experiments as a *punctipennis pool,* was formed by the receding creek mentioned above and fed by seepage and a trickling connection with the main stream. It was shaded, cool and thickly overhung with surrounding brush, mainly grape, cottonwood and sycamore. The bottom was made up of water-rounded stones ranging in size from pebbles to small boulders and its prominent vegetable growth was a member of the Crenothrix group. The water was unusually clear and had an alkalinity of 840 parts of $CaCO_3$ per

million. It showed evidence of being permanent, in part at least, throughout the summer. Two boxes were installed as mentioned above (Fig. 2) enclosing a section in which larvae had been observed and removed. On June 28, 474 eggs of *A. quadrimaculatus* were placed in one box and on July 1, 635 eggs of *A. punctipennis* were placed in the other. These eggs were observed to hatch in the normal period in both boxes and daily observations proved their gradual development, pupation beginning on the thirteenth day after egg deposition. No accurate count of the numbers emerging was possible under the existing conditions, but from general observation, no retardation in development or diminution in the expected numbers of *A. quadrimaculatus,* altho breeding in an *A. punctipennis* pool, could be noted. The results of this experiment left to our minds only two alternatives in the question of selective breeding places—either the *punctipennis* larvae are cannibalistic on the *quadrimaculatus* larvae when the former are in optimum surroundings, and the process is reversed when optimum conditions are furnished to *quadrimaculatus* larvae, or what is far more likely—the female exercises selection in depositing her eggs. Several experiments were inaugurated to settle this first alternative by mixing the eggs in one box but the floods mentioned above rendered our results untrustworthy.

Incubation Period.—In the regular routine of laboratory work, the jars were examined every morning at about nine o'clock, the eggs that were found for the first time being entered as deposited for that day and those found to be hatched were entered at the same time. By observing this routine the average incubation period was very nearly approximated. For *A. quadrimaculatus* the average incubation period was 2.5 days with a range from 2 to 4 days. The eggs of *A. punctipennis* averaged 3.2 days with a range from 2 to 6 days. Temperature is quoted by many authors as distinctly influencing the length of the incubation period. With many of our sets, however, laid on the same day and subjected to the same conditions a considerable amount of variation was recorded. It seems highly probable that temperature should exercise a decided effect on incubation particularly at the extremes but within a certain range such as our eggs were subjected to, i. e., 68° to 76° F., little effect could be noted.

Desiccation Experiments.—Mitchell (1907) states that Dr. Dupree "has had the eggs of *Anopheles* develop after as many as ten hours out of water, this, however, being exceptional." Stephens and Christophers (1908) state anopheline eggs removed from water, placed on paper and allowed to dry for more than two, or, at the most three days, will not hatch if they are kept at a temperature of 86° to 96° F. In an attempt to check these findings for the Californian species, eggs

of *A. quadrimaculatus* and *A. punctipennis* were removed from the water six hours after they had been deposited by allowing them to adhere to the surface of a strip of filter paper dipped among them, leaving a number of eggs in the jar as a check. The filter paper was then suspended by pins inside a capsule box and allowed to dry out at room temperature. Drying was accomplished in a remarkably short time for at the end of four hours the paper was entirely dry and the eggs rattled off its surface at the least movement. At intervals of twenty-four hours a supply of dried eggs were taken from the filter paper and placed in shell vials of tap water. We were never able to obtain a hatch from eggs of *A. punctipennis* that had been removed from water for twenty-four hours. However, with *A. quadrimaculatus* eggs removed from the water on June 14, dried and replaced on the fifteenth, sixteenth and seventeenth, having been subjected to drying for 24, 48 and 72 hours, respectively, there were produced excellent hatches on the seventeenth, eighteenth and nineteenth, showing not only that the eggs of this species can withstand drying for these periods, but also the rather interesting fact that egg development ceases as soon as they are removed from the water. Eggs from this lot placed in water on the eighteenth (96 hours of drying) and for several succeeding days failed to hatch. The maximum and minimum temperatures to which the eggs were subjected during this period were respectively 74° F. and 65° F. Another attempt to duplicate this set of experiments with the same species and technique when the temperature ranged between 70° F. and 80° F. resulted in the failure of the eggs to hatch after 48 hours of drying.

The authors present this paper not as a complete treatise on egg deposition of Anophelines but as observations that may add to the general fund of information concerning these insects whose activities are of such vital interest and importance to mankind in all parts of the world. The authors wish to acknowledge the helpful co-operation and limitless enthusiasm in this work on the part of their two student assistants, Mr. Clifford T. Dodds and Mr. John F. Lamiman of the University of California.

SUMMARY

1. The process of egg deposition in *Anopheles punctipennis* is described.

2. The number of eggs deposited per laying is found to be greater than hitherto recorded, *A. quadrimaculatus* averaging 209 eggs and *A. punctipennis* 203 per laying.

3. Descriptions are given of the eggs of the Californian anophelines whereby they may be differentiated, including a description of the

egg of *A. pseudopunctipennis,* which represents a marked departure from the usual anopheline type.

4. Observations are introduced to indicate that specific breeding places are due to selective oviposition.

5. The incubation period of the eggs of *A. quadrimaculatus* is 2.5 days, and *A. punctipennis* 3.2 days, and *A. pseudopunctipennis,* 3 days.

6. It was found that the eggs of *A. quadrimaculatus* could withstand drying for 72 hours but that those of *A. punctipennis* failed to hatch after 24 hours of drying.

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